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(54) Title: BIOFLAVONOIDS AS PLASMA HIGH DENSITY LIPOPROTEIN LEVEL INCREASING AGENT		
<div style="text-align: center;"> </div> <div style="text-align: right;">(I)</div>		
(57) Abstract <p>A use of a bioflavonoid of formula (I) or plant extract containing same for increasing the plasma high density lipoprotein (HDL) level in a mammal, wherein, R¹, R², R³, R⁴, R⁵, R⁶, R⁷, R⁸ and R⁹ are each independently hydrogen; a hydroxy group; a C₁₋₉ alkoxy group optionally substituted with one or more substituents selected from the group consisting of a hydroxy, C₁₋₅ alkoxy, aryloxy, and phenyl group substituted with 1 to 3 substituents selected from the group consisting of a hydroxy, alkoxy, aryloxy, halogen, nitro and amide group; a C₅₋₉ cycloalkyloxy group substituted with 1 to 3 substituents selected from the group consisting of a hydroxy, alkoxy, aryloxy, halogen, nitro and amido group; a C₅₋₉ cycloalkylcarbonyloxy group substituted with 1 to 3 substituents selected from the group consisting of a hydroxy, alkoxy, aryloxy, halogen, nitro and amido group; a C₂₋₁₀ or C₁₆₋₁₈ acyloxy group optionally substituted with one or more substituents selected from the group consisting of a hydroxy, C₁₋₅ alkoxy, aryloxy, and phenyl group substituted with 1 to 3 substituents selected from the group consisting of a hydroxy, alkoxy, aryloxy, halogen and nitro group; a rutinosyl group; or a rhaminosyl group; and X is a single or double bond.</p>		

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**BIOFLAVONOIDS AS PLASMA HIGH DENSITY LIPOPROTEIN LEVEL
INCREASING AGENT**

FIELD OF THE INVENTION

5

The present invention relates to a use of a bioflavonoid for increasing the plasma high density lipoprotein(HDL) level in a mammal.

10 BACKGROUND OF THE INVENTION

In recent years, coronary cardio-circulatory diseases, e.g., atherosclerosis and hypercholesterolemia, have increasingly become a major cause of deaths. It has been reported that an elevated plasma cholesterol level causes the deposition of fat, macrophages and foam cells on the wall of blood vessels, such deposit leading to plaque formation and then to atherosclerosis(Ross, R., Nature, 362, 801-809(1993)).

20 Specifically, it has been reported that a high ratio of plasma low density lipoproteins(LDL) to total cholesterol causes atherosclerosis very easily, while plasma HDL-cholesterol is beneficial to health. A recent study exhibited that increase in the plasma HDL level is inversely related to the occurrence of a heart disease(Barter P. J., Rye K. A., High density lipoproteins and coronary heart disease, Atherosclerosis 121:1-12(1996)).

25 Lamarche et al. have found that a combination of hypertriglyceridemia, a low plasma HDL level, an abdominal fatness and the like is a major risk factor causing atherosclerosis, thereby discovering that a low plasma HDL level is also an important risk factor of atherosclerosis (Lamarche B., Lewis G. F., Atherosclerosis prevention for the next decade: risk assessment beyond low density lipoprotein cholesterol, Can. J. Cardiol. 14:841-851(1998)).

30 In addition, Lacko et al. have verified that plasma HDL has anti-inflammatory and anti-atherosclerosis activities(Lacko

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A. G., Miller N. E., International symposium on the role of HDL in disease prevention: report on a meeting, J. Lipid Research 38:1267-1273(1997)).

Therefore, numerous efforts have been made to develop medicines to increase the plasma HDL level; and, as a result, a pharmaceutical composition for increasing the plasma HDL level has been reported(US Patent No. 5,783,600, issued on July 21,1998). However, said composition comprises chemically synthesized compounds as an active ingredient, which may induce adverse side effects in a human body in terms of toxicity or pharmaceutical activities.

The present inventors have endeavored to develop a non-toxic plasma HDL level increasing agent from natural materials, and, as a result, have discovered that bioflavonoids isolated from edible plants are effective to increase the plasma HDL level.

Generally, various bioflavonoids, such as those listed in Table I, are present in the citrus peel(Horowitz, R. M. et al., J. Org. Chem., 25, 2183-2187(1960)). Hesperidin is the major bioflavonoid component found in orange, lemon and tangerine; naringin represents the major bioflavonoid component in grapefruit; and naringin and hesperidin are present in citron in nearly equal amounts.

Table I

Citrus fruit	Bioflavonoids
Grapefruit	apigenin, dihydrokaempferol, eriodictyol, hesperetin, hesperidin, isorhamnetin, isosakuranetin, neohesperidin, poncirin, quercetin, rutin

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Lemon	apigenin, apigenin 7-rutinoside, chrysoeriol, diosmin, eriocitrin, hesperidin, isorhamnetin, limocitrin, limocitrol, luteolin 7-rutinoside, naringin, neohesperidin, poncirin, quercetin
Orange	auranetin, hesperidin, isosakuranetin 7-rutinoside, naringin, neohesperidin, nobiletin, rutin, sinensetin, tangeretin, vitexin
Tangerine	hesperidin, nobiletin, tangeretin

5 It has been reported that the bioflavonoids isolated from citrus peel have an anti-oxidative, anti-cancer, anti-viral and blood-pressure lowering activities(Saija, A., et al., Free Radical Biol. Med., 19, 481-486(1995); Matsubara, Y., et al., Japan Organic Synthesis Chem. Association
10 Journal, 52, 318-327(1994, Mar.); Galati, E. M., et al., Farmaco., 51(3), 219-221(1996, Mar.); Felicia, V., et al., Nutr. Cancer, 26, 167-181(1996); EP 0352147 A2(1990. 1. 24); and Kaul, T. N., et al., J. Med. Biol., 15, 71-75(1985)).

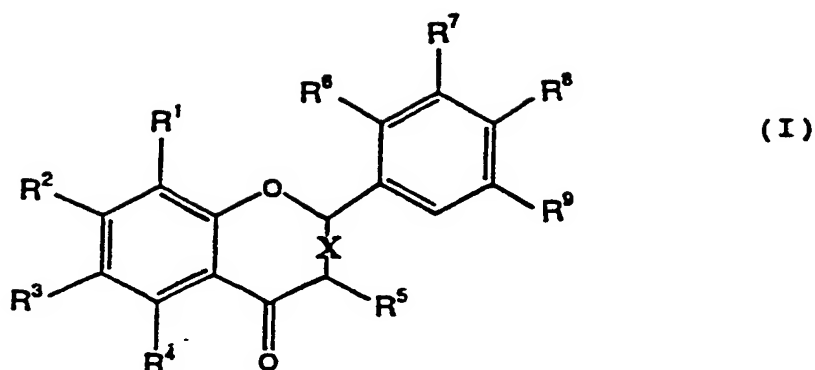
15 However, bioflavonoids have never been reported to have plasma HDL level increasing activity.

SUMMARY OF THE INVENTION

20 Accordingly, it is a primary object of the present invention to provide a use of a bioflavonoid for increasing the plasma HDL level in a mammal.

25 In accordance with the present invention, there is provided a use of a bioflavonoid of formula(I) or a plant extract containing same for increasing the plasma HDL level in a mammal:

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wherein

10 R^1 , R^2 , R^3 , R^4 , R^5 , R^6 , R^7 , R^8 and R^9 are each independently hydrogen; a hydroxy group; a C_{1-9} alkoxy group optionally substituted with one or more substituents selected from the group consisting of a hydroxy, C_{1-5} alkoxy, aryloxy, and phenyl group substituted with 1 to 3
15 substituents selected from the group consisting of a hydroxy, alkoxy, aryloxy, halogen, nitro and amido group; a C_{5-9} cycloalkyloxy group substituted with 1 to 3 substituents selected from the group consisting of a hydroxy, alkoxy, aryloxy, halogen, nitro and amido group; a C_{5-9}
20 cycloalkylcarbonyloxy group substituted with 1 to 3 substituents selected from the group consisting of a hydroxy, alkoxy, aryloxy, halogen, nitro and amido group; a C_{2-10} or C_{16-18} acyloxy group optionally substituted with one or more substituents selected from the group consisting of
25 a hydroxy, C_{1-5} alkoxy, aryloxy, and phenyl group substituted with 1 to 3 substituents selected from the group consisting of a hydroxy, alkoxy, aryloxy, halogen and nitro group; a rutinosyl group; or a rhaminosyl group; and
X is a single or double bond.

Detailed Description of the Invention

Among the bioflavonoids of the present invention, preferred are those of formula(I) wherein: R^1 is H; R^2 is OH,
35 a rutinosyl or rhaminosyl group; R^3 is H; R^4 is OH; R^5 is H, OH or a rutinosyl group; R^6 is H; R^7 is H or OH; R^8 is OH or OCH_3 ; and R^9 is H.

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Particularly preferred bioflavonoids of formula(I) of the present invention are shown in Table II.

Table II

	R ¹	R ²	R ³	R ⁴	R ⁵	R ⁶	R ⁷	R ⁸	R ⁹	X
5 Eridictyol	H	OH	H	OH	H	H	OH	OH	H	single bond
Hesperidin	H	ORut	H	OH	H	H	OH	OCH ₃	H	single bond
Hesperetin	H	OH	H	OH	H	H	OH	OCH ₃	H	single bond
Naringin	H	ORha	H	OH	H	H	H	OH	H	single bond
Naringenin	H	OH	H	OH	H	H	H	OH	H	single bond
10 Apigenin	H	OH	H	OH	H	H	H	OH	H	double bond
Luteolin	H	OH	H	OH	H	H	OH	OH	H	double bond
Diosmin	H	ORut	H	OH	H	H	OH	OCH ₃	H	double bond
Kaemferol	H	OH	H	OH	OH	H	H	OH	H	double bond
Quercetin	H	OH	H	OH	OH	H	OH	OH	H	double bond
15 Rutin	H	OH	H	OH	ORut	H	OH	OH	H	double bond

note) ORut : Rutinosyl group

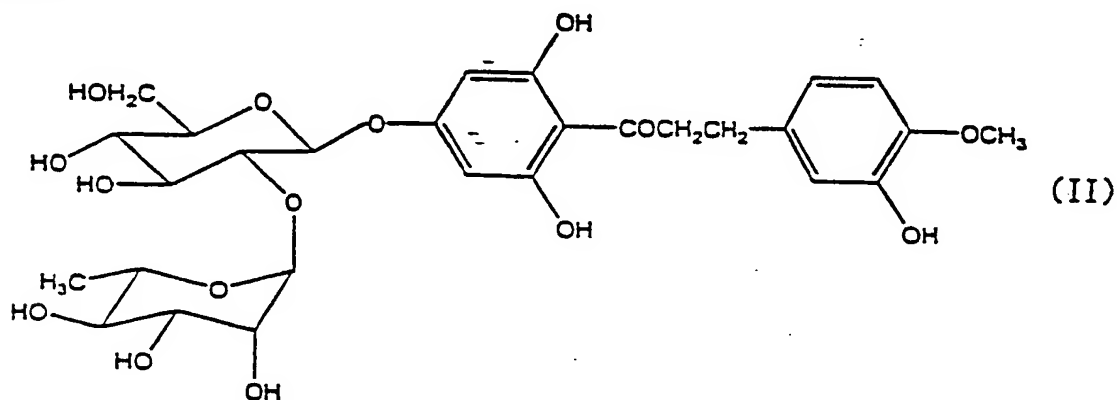
ORha : Rhaminosyl group

20 The bioflavonoids of the present invention may be extracted from various plants including vegetables such as lettuce and onion, fruits such as citrus fruit, and grains such as buckwheat, or synthesized in accordance with the conventional process described by Zemplen, Bogнар in Ber.,
 25 1043(1943) and Seka, Prosche, Monatsh., 69, 284(1936). For example, rutin and quercetin may be extracted from buckwheat by using a suitable solvent such as water or aqueous alcohol under a high temperature and pressure. Alternatively, buckwheat seeds may be allowed to stand overnight in an
 30 aqueous solution of Ca(OH)₂ or NaOH, and then crude rutin precipitates may be collected after neutralization.

Further, dry powders of buckwheat seeds, leaves, stems and flowers may also be used. Generally, the content of rutin in leaves and stems of buckwheat is about 0.6% and that in buckwheat flower is about 3%.

5 The citrus which can be used in the present invention may be tangerine, orange, lemon, grapefruit and citron. It is preferable to use the peel of citrus fruits uncontaminated by chemical pesticides. The citrus peel extract may be prepared by any of the conventional methods using water or other suitable solvents such as aqueous
10 alcohol, $\text{Ca}(\text{OH})_2$ and NaOH .

On the other hand, neohesperidin dihydrochalcone ($\text{C}_{28}\text{H}_{36}\text{O}_{15}$) of formula(II), which can be easily derived from naringin and has a 1,000 to 1,500 fold higher sweetness than
15 sucrose, may also be used for increasing the plasma HDL level:



Bioflavonoids of formula(I) and (II) start to exert a plasma HDL level increasing effect at a dose of only 0.1 mg/kg/day, the effect increasing with the dose thereof.

Moreover, in spite of their potent efficacies, the
30 bioflavonoid and plant extract containing same show little toxicity or mitogenicity in tests using mice. More specifically, naringin, naringenin, hesperidin, hesperetin, diosmin, neohesperidin dihydrochalcone, quercetin or rutin exhibits no toxicity when it is orally administered to a
35 mouse at a dose of 1,000 mg/kg. Further, the bioflavonoid or the citrus peel extract exerts no adverse effects on the liver function.

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The present invention also provides a pharmaceutical composition for increasing the plasma HDL level, which comprise the bioflavonoid or the plant extract containing same as an active ingredient and pharmaceutically acceptable excipients, carriers or diluents.

A pharmaceutical formulation may be prepared in accordance with any of the conventional procedures. In preparing the formulation, the active ingredient is preferably admixed or diluted with a carrier, or enclosed within a carrier which may be in the form of a capsule, sachet or other container. When the carrier serves as a diluent, it may be a solid, semi-solid or liquid material acting as a vehicle, excipient or medium for the active ingredient. Thus, the formulations may be in the form of a tablet, pill, powder, sachet, elixir, suspension, emulsion, solution, syrup, aerosol, soft and hard gelatin capsule, sterile injectable solution, sterile packaged powder and the like.

Examples of suitable carriers, excipients, and diluents are lactose, dextrose, sucrose, sorbitol, mannitol, starches, gum acacia, alginates, gelatin, calcium phosphate, calcium silicate, cellulose, methyl cellulose, microcrystalline cellulose, polyvinylpyrrolidone, water, methylhydroxybenzoates, propylhydroxybenzoates, talc, magnesium stearate and mineral oil. The formulations may additionally include fillers, anti-agglutinating agents, lubricating agents, wetting agents, flavoring agents, emulsifiers, preservatives and the like. The compositions of the invention may be formulated so as to provide quick, sustained or delayed release of the active ingredient after their administration to a mammal by employing any of the procedures well known in the art.

The pharmaceutical composition of the present invention can be administered via various routes including oral, transdermal, subcutaneous, intravenous and intramuscular introduction. In case of human, a typical daily dose of the bioflavonoid may range from about 0.1 to 500 mg/kg body

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weight, preferably 0.5 to 100 mg/kg body weight, and can be administered in a single dose or in divided doses.

However, it should be understood that the amount of the active ingredient actually administered ought to be determined in light of various relevant factors including the condition to be treated, the chosen route of administration, the age, sex and body weight of the individual patient, and the severity of the patient's symptom; and, therefore, the above dose should not be intended to limit the scope of the invention in any way.

Moreover, the bioflavonoid or the plant extract containing same in the form of an additive or a dietary supplement can be incorporated in foods or beverages for the purpose of increasing the plasma HDL level. The foods or beverages may include meats; juices such as a vegetable juice(e.g., carrot juice and tomato juice) and a fruit juice(e.g., orange juice, grape juice, pineapple juice, apple juice and banana juice); chocolates; snacks; confectionery; pizza; foods made from cereal flour such as breads, cakes, crackers, cookies, biscuits, noodles and the likes; gums; dairy products such as milk, cheese, yogurt and ice creams; soups; broths; pastes, ketchups and sauces; teas; alcoholic beverages; carbonated beverages such as Coca-Cola® and Pepsi-Cola®; vitamin complexes; and various health foods.

In this case, the content of the bioflavonoid in a food or beverage may range from 0.01 to 50% by weight, preferably 0.05 to 10% by weight. In particular, the beverage according to the present invention may comprise 200 to 10,000 mg of the bioflavonoid per 1000 ml of the beverage. In case of plant powder, the content thereof in a food or beverage may range from 0.5 to 30% by weight.

As described above, a bioflavonoid or a plant extract containing same can be used as an effective, non-toxic pharmaceutical agent for increasing the plasma HDL level.

The following Examples are intended to further illustrate the present invention without limiting its scope.

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Further, percentages given below for solid in solid mixture, liquid in liquid, and solid in liquid are on a wt/wt, vol/vol and wt/vol basis, respectively, and all the reactions were carried out at room temperature, unless specifically indicated otherwise.

Example 1: Toxicity of Orally Administered Rutin

12 seven-week-old specific pathogen-free ICR mice, six female mice each weighing about 25 to 29 g and six male mice each weighing about 34 to 38 g, were bred under an environment of $22 \pm 1^\circ\text{C}$, $55 \pm 5\%$ relative humidity and 12L/12D photoperiod. Fodder (Cheiljedang Co., mouse and rat fodder) and water were sterilized and fed to the mice.

Rutin (Sigma Chemical Co., St. Louis, Mo., U.S.A.) was dissolved in 0.5% Tween 80 to a concentration of 100 mg/ml, and the solution was orally administered to the mice in an amount of 0.2 ml per 20 g of mouse body weight. The solution was administered once and the mice were observed for 10 days for signs of adverse effects or death according to the following schedule: 1, 4, 8, and 12 hours after the administration and, every 12 hours thereafter. The weight changes of the mice were recorded every day to examine the effect of rutin. Further, on the 10th day, the mice were sacrificed and the internal organs were visually examined.

All the mice were alive at day 10 and rutin showed no toxicity at a dose of 1,000 mg/kg. The autopsy revealed that the mice did not develop any pathological abnormality, and no weight loss was observed during the 10 day test period. Accordingly, it was concluded that rutin is not toxic when orally administered to an animal.

Example 2: Administration of Bioflavonoids to an Animal-(1)

(Step 1) Animal Test

40 three-week-old Sprague-Dawley rats (Taihan laboratory animal center, Korea) each weighing about 90 to 110 g were

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evenly divided into four dietary groups by a randomized block design. The rats of the four groups were fed with four different high-cholesterol diets, i.e., AIN-76 laboratory animal diet(ICN Biochemicals, Cleveland, OH, U.S.A.) containing 1% cholesterol(Control group), 1% cholesterol plus 0.1% hesperetin(Hesperetin group), 1% cholesterol plus 0.1% naringin(Naringin group) and 1% cholesterol plus 16.7% citrus peel extract(Citrus peel extract group), respectively. The compositions of diets fed to the four groups are shown in Table III.

Table III

Dietary group	Control group	Hesperetin group	Naringin group	Citrus peel extract ^{*4} group
Ingredients				
Casein	20	20	20	20
D,L-methionine	0.3	0.3	0.3	0.3
Corn starch	15	15	15	15
Sucrose	49	48.9	48.9	32.3
Cellulose powder ^{*1}	5	5	5	5
Mineral mixture ^{*1}	3.5	3.5	3.5	3.5
Vitamin mixture ^{*1}	1	1	1	1
Choline bitartrate	0.2	0.2	0.2	0.2
Corn oil	5	5	5	5
Cholesterol	1	1	-	1
Hesperetin ^{*2}	-	0.1	-	-
Naringin ^{*2}	-	-	0.1	-
Citrus peel extract ^{*3}	-	-	-	16.7
Total	100	100	100	100

^{*1}: Purchased from TEKLAD Premier Co.(Madison, WI, U.S.A.).

^{*2}: Purchased from Sigma Chemical Company(St. Louis, Mo., U.S.A.)

^{*3}: Prepared by extracting the tangerin peel in 30% ethanol during 3 hours, followed by concentrating the resulting extract by vacuum

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*4: 0.1% hesperidin equivalent

The rats were allowed to feed freely on the specified diet together with water for eight weeks, the ingestion amount was recorded daily and the rats were weighed every 7 days, and then the record was analyzed. All rats showed a normal growth rate and there was observed no significant difference among the three groups in terms of the feed ingestion amount and the weight gain.

(Step 2) Blood Analysis

The effect of administering bioflavonoids to rats on the plasma cholesterol was determined as follows.

Blood samples were taken from the rats of the four dietary groups and plasma HDL fractions were separated therefrom by using the method of adding a HDL-cholesterol reagent(Chiron Diagnostics Co., USA) containing dextran-sulfate to the plasma from the blood sample in a ratio of reagent:plasma=1:10, reacting the mixture in an incubator for 5 minutes and, then, centrifuging the resulting mixture on a speed of 2,500 rpm for 10 minutes(Stein, E. A., et al., Clin. Chem., 24: 1112-1115(1978); Finley, P. R., et al., Clin. Chem., 24: 931-933(1978); Warnick, G. R., et al., Clin. Chem., 28: 1379-1388(1982)). Total cholesterol and HDL-cholesterol levels were determined by using a Blood Chemical Analyzer(Ciba Corning 550 Express, USA). The result is shown in Table IV, wherein the ratio of HDL-cholesterol/total-cholesterol level increased by 27, 52 and 67% in the hesperetin, naringin and citrus peel extract groups, respectively, as compared with that of the control group.

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Table IV

Group	Control group	Hespere-tin group	Naringin group	Citrus peel extract group
Lipids Conc.				
Total-C (mg/dl)	147.11±11	125.1±16.1	100.8±16.1	94.5±12
HDL-C (mg/dl)	22.2±2.1	25.7±1.5	24.0±1.5	24.8±1.0
HDL-C (%)	15.7±1.6	20.0±1.9	23.9±3.1	26.2±7.5
Total-C				

* Total-C: Total-cholesterol

* HDL-C: HDL-cholesterol

Example 3: Administration of Bioflavonoids to an Animal- (2)

(Step 1) Animal Test

34 four-week-old male Sprague-Dawley rats(Taihan laboratory animal center, Korea) each weighing about 110 to 130 g were evenly divided into four dietary groups by a randomized block design. The rats of the four groups were fed with four different diets, i.e., test diet 5799M-B(PMI, U.S.A.) containing 1% cholesterol and 20% lard(Control group); 1% cholesterol and 20% lard plus 0.1% diosmin(Diosmin group); 1% cholesterol and 20% lard plus 0.05% neohesperidin dihydrochalcone(Neohesperidin group); and 1% cholesterol and 20% lard plus 0.1% rutin(Rutin group), respectively. Test diet 5799M-B comprises 21% vitamin free casein, 15% sucrose, 3% cellulose, 2% vitamin mixture, 5% mineral mixture, 0.15% D,L-methionine, 0.5% sodium cholate, 32.15% dextrin, 20% lard, 0.2% choline chloride and 1% cholesterol. 8 or 9 rats were allotted to each group and diosmin, neohesperidin dihydrochalcone and rutin were purchased from Sigma Chemical Company(St. Louis, Mo., U.S.A.). The rats were bred for 6 weeks while

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being allowed free access to the diets and water.

(Step 2) Blood Analysis

The effect of administering bioflavonoids to rats on the plasma cholesterol was determined as follows.

Blood samples were taken from the rats and total cholesterol and HDL-cholesterol levels were determined in accordance with the same procedure in (Step 2) of Example 2. The result is shown in Table V.

Table V

Group Lipids Conc.	Control group	Diosmin group	Neohesperidin group	Rutin group
Total-C (mg/dl)	690	503	336	373
HDL-C (mg/dl)	70±19	131±59	180±90	216±11
HDL-C Total-C (%)	10	26	53	58

* Total-C: Total-cholesterol

* HDL-C: HDL-cholesterol

As can be seen from Table V, bioflavonoids of the present invention increase the plasma HDL remarkably in an animal and, thereby, suppressing the onset of cardiovascular diseases.

Example 4: Administration of Bioflavonoids to Man

Two men in their fifties were treated with daily oral dosage of 10 mg/kg of naringin and hesperidin, respectively, for 2 months. The plasma HDL level was determined before and after the administration. The result is shown in Table VI.

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Table VI. Average plasma HDL level(mg/dl)

Group	Before Administration	After 2 months	Increase rate (%)
Naringin	51	60	18
Hesperidin	47	56	19

Consequently, naringin and hesperidin increased the plasma HDL level by 18% and 19%, respectively, in comparison to that before the administration.

Example 5: HDL increasing agent

Hard gelatin capsules were prepared using the following ingredients:

	Quantity (mg/capsule)
Active ingredient(bioflavonoids)	200
Vitamin C	50
<u>Lactose(carrier)</u>	<u>150</u>
Total	400 mg

Example 6: Foods containing Bioflavonoids

(1) Preparation of tomato ketchup and sauce

Naringin was added to a tomato ketchup or sauce in an amount ranging from 0.01 to 50 wt% to obtain a health-improving tomato ketchup or sauce.

(2) Preparation of wheat flour foods

Rutin was added to a wheat flour in an amount ranging from 0.01 to 50 wt% and breads, cakes, cookies, crackers and noodles were prepared by using the mixture to obtain health-improving foods.

(3) Preparation of soups and gravies

Quercetin was added to soups or gravies in an amount ranging from 0.01 to 50 wt% to obtain a health-improving

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soups or gravies.

(4) Preparation of ground beef

Diosmin was added to ground beef in an amount ranging
5 from 0.01 to 50 wt% to obtain a health-improving ground
beef.

(5) Preparation of dairy product

Rutin or Quercetin was added to milk in an amount
10 ranging from 0.01 to 50 wt% to obtain a health-improving
milk.

Especially, in case of cheese preparation, rutin or
quercetin was added to the coagulated milk protein, and in
case of yogurt preparation, rutin or quercetin was added to
15 the coagulated milk protein after the fermentation.

Example 7: Beverages containing Bioflavonoids

(1) Preparation of vegetable juice

20 200 to 10,000 mg of hesperidin was added to 1000 ml of
a tomato or carrot juice to obtain a health-improving
vegetable juice.

(2) Preparation of fruit juice

25 200 to 10,000 mg of hesperidin was added to 1000 ml of
an apple or grape juice to obtain a health-improving fruit
juice.

(3) Preparation of carbonated drink

30 20 to 10,000 mg of hesperidin was added to 1000 ml of
Coca Cola® and Pepsi Cola® to obtain a health-improving
carbonated juice.

Example 8: Health foods containing Bioflavonoids

35

(1) A health food was prepared by mixing the following
ingredients and tableting the mixture.

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	Quantity (wt/wt %)
Naringin, hesperidin or a plant extract containing it	5
5 Ginseng powder or extract	20
<u>Sweetner and flavour</u>	<u>75</u>
Total	100

10 (2) Preparation of buckwheat powder and Extraction of
rutin from buckwheat

Buckwheat seeds, leaves, stems and flowers were dried
at a room temperature and then powdered.

15 Alternatively, 100 g each of buckwheat leaves and
flowers was extracted twice with 200 ml each of 70% ethanol
at 40 °C for 5 hours. The extracts thus obtained were
filtered. The resulting extracts had 1.8% and 4% of rutin,
respectively.

20 In addition, $\text{Ca}(\text{OH})_2$ was added to buckwheat leaves or
flowers to pH 12.0 and the mixture was allowed to stand
overnight. The mixture was adjusted to pH 6 to 7 and the
resulting precipitate was recovered to obtain a crude
rutin(purity: 40 to 50%).

25 A medicine or health food containing the rutin powder
or extract thus obtained may be prepared in accordance with
a conventional method.

(3) A mixture containing the following ingredients was
prepared:

	Quantity (wt/wt %)
30 Onion powder	40
Garlic powder	10
Jujube powder	30
Buckwheat flower powder	5
35 <u>Dry grape powder</u>	<u>15</u>
Total	100

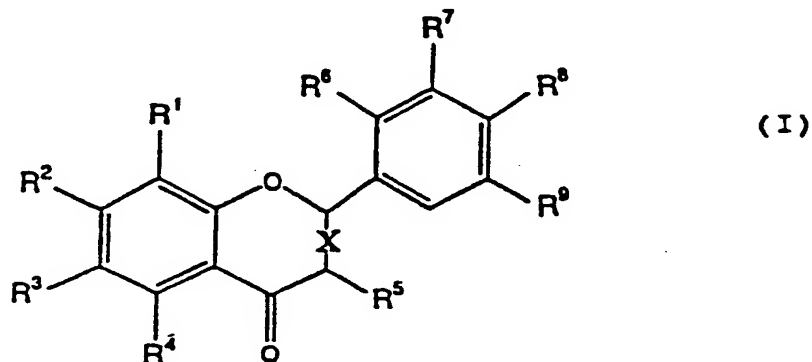
- 17 -

The mixture was added to a conventional fodder in an amount of 5 wt% and the resulting fodder was fed to rats for 2 months. Consequently, the plasma HDL level of rats after 2 months increased by 30% as average in comparison with that of rats just before the administration.

While the invention has been described with respect to the above specific embodiments, it should be recognized that various modifications and changes may be made to the invention by those skilled in the art which also fall within the scope of the invention as defined by the appended claims.

What is claimed is:

1. A use of a bioflavonoid of formula (I) or a plant extract containing same for increasing the plasma high density lipoprotein(HDL) level in a mammal:



15 wherein,

R^1 , R^2 , R^3 , R^4 , R^5 , R^6 , R^7 , R^8 and R^9 are each independently hydrogen; a hydroxy group; a C_{1-9} alkoxy group optionally substituted with one or more substituents selected from the group consisting of a hydroxy, C_{1-5} alkoxy, aryloxy, and phenyl group substituted with 1 to 3 substituents selected from the group consisting of a hydroxy, alkoxy, aryloxy, halogen, nitro and amido group; a C_{5-9} cycloalkyloxy group substituted with 1 to 3 substituents selected from the group consisting of a hydroxy, alkoxy, aryloxy, halogen, nitro and amido group; a C_{5-9} cycloalkylcarbonyloxy group substituted with 1 to 3 substituents selected from the group consisting of a hydroxy, alkoxy, aryloxy, halogen, nitro and amido group; a C_{2-10} or C_{16-18} acyloxy group optionally substituted with one or more substituents selected from the group consisting of a hydroxy, C_{1-5} alkoxy, aryloxy, and phenyl group substituted with 1 to 3 substituents selected from the group consisting of a hydroxy, alkoxy, aryloxy, halogen and nitro group; a rutinosyl group; or a rhaminosyl group; and

35 X is a single or double bond.

2. The use of claim 1, wherein the mammal is human.

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3. The use of claim 1, wherein the bioflavonoid is hesperidin, hesperetin, naringin, naringenin, diosmin, rutin, quercetin or a mixture thereof.

4. The use of claim 1, wherein the plant extract is a vegetable or fruit extract.

5. The use of claim 4, wherein the plant extract is an extract of buckwheat sprouts, seeds, leaves, stems or flowers.

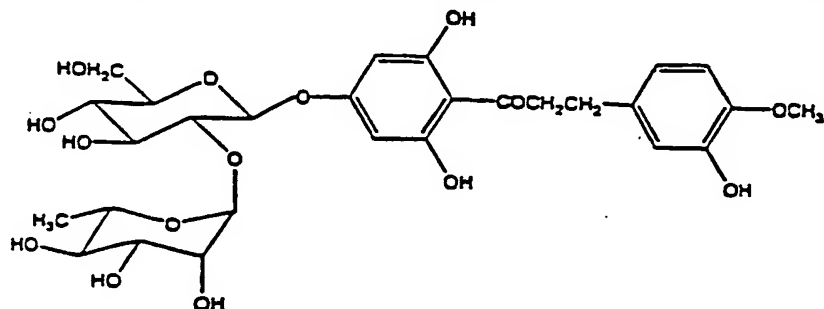
6. The use of claim 1, wherein the bioflavonoid or plant extract is administered to the mammal in the form of a composition containing same, said composition being selected from the group consisting of: a pharmaceutical composition, a food composition and a beverage composition.

7. The use of claim 6, wherein the effective amount of the bioflavonoid contained in the pharmaceutical composition ranges from 0.5 to 100 mg/kg body weight/day.

8. The use of claim 6, wherein the content of the bioflavonoid in the food composition ranges from 0.01 to 50% by weight.

9. The use of claim 6, wherein the content of the bioflavonoid in the beverage composition ranges from 200 to 10,000 mg per 1,000 ml of the beverage.

10. A use of neohesperidin dihydrochalcone of formula (II) for increasing the plasma HDL level in a mammal:



(II)

INTERNATIONAL SEARCH REPORT

International application No.

PCT/KR 98/00326

A. CLASSIFICATION OF SUBJECT MATTER

IPC⁷: A 61 K 31/353, A 61 K 35/78

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC⁷: A 61 K 31/353, A 61 K 35/78

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

WPI, CAS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	Database WPIL on Questel, week 9702, London: Derwent Publications Ltd., AN 97-017298, Class A61K, JP 08-283154 A (NIPPON SHINYAKU CO., LTD.) 29 October 1996 (29.10.96), abstract.	1-9
X	Database WPIL on Questel, week 9702, London: Derwent Publications Ltd., AN 97-014823, Class A61K, JP 08-280358 A (NIPPON SHINYAKU CO., LTD.) 29 October 1996 (29.10.96), abstract.	1-9
X	Chem. abstr., Vol.103, No.25, 23. Dezember 1985 (Columbus, OH, USA), page 72, column 1, abstract No. 206002f, SYROV, V. N. et al. "Prospects for the study of flavonoids as hypolipidemic and antiatherosclerotic agents", Dokl. Akad. Nauk UzSSR 1985, (3), 48-50 (Russ).	1-9

☐ Further documents are listed in the continuation of Box C.☒ See patent family annex.

* Special categories of cited documents:

„A“ document defining the general state of the art which is not considered to be of particular relevance

„E“ earlier application or patent but published on or after the international filing date

„L“ document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

„O“ document referring to an oral disclosure, use, exhibition or other means

„P“ document published prior to the international filing date but later than the priority date claimed

„T“ later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

„X“ document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

„Y“ document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

„&“ document member of the same patent family

Date of the actual completion of the international search

26 March 2000 (26.03.00)

Date of mailing of the international search report

29 March 2000 (29.03.00)

Name and mailing address of the ISA/AT
Austrian Patent Office
Kohlmarkt 8-10; A-1014 Vienna
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Form PCT/ISA/210 (second sheet) (July 1998)

INTERNATIONAL SEARCH REPORT

International Application No.

PCT/KR 98/00326

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	Chem. abstr., Vol.70, No.11, 17. March 1969 (Columbus, OH, USA), page 189, column 2, abstract.No.45961a, LISEVITSKAYA, L.T. et al. "Action of quercetin and flavonoid preparations from Pontic azalea (Rhododendron luteum) and Caucasian rhododendron (R. caucasicum) on some indexes of cholesterol metabolism in white rats with experimental hypercholesteremia, " Biol. Nauki 1968 (12), 50-2 (Russ).	1-9
X	Chem. abstr. Vol.122, No.23, 5. June 1995 (Columbus, OH, USA), page 94, column 1, abstract No.281935h, IGARASHI, K. et al. "Effects of isorhamnetin, rhamnetin, and quercetin on the concentrations of cholesterol and lipoperoxide in the serum and liver and on the blood and liver antioxidative enzyme activities of rats," Biosci., Biotechnol., Biochem. 1995, 59(4), 595-601 (Eng).	1-9
X	Chem. abstr. Vol.76, No.19, 08 May 1972 (Columbus, OH, USA), page 37, column 2, abstract No.108080j, LISEVITSKAYA, L.I. et al. "Effect of luteoline and luteoline-7-glycoside on lipid metabolism during experimental atherosclerosis", Aktual. Vop. Farm. 1968 (Pub. 1970), 178-9 (Russ).	1-9
X	Chem. abstr. Vol.76, No.19, 08 May 1972 (Columbus, OH, USA), page 37, column 2, abstract No.108079r, LISEVITSKAYA, L.I. et al. "Flavanol substances during experimental atherosclerosis", Aktual. Vop. Farm. 1968 (Pub. 1970), 176-7 (Russ).	1-9
X	Database WPIL on Questel, week 9518, London: Derwent Publications Ltd., AN 95-136792, Class A23L; & JP 07-061927 A (LOTTE CO LTD), abstract.	1-9
X	Database WPIL on Questel, week 9251, London: Derwent Publications Ltd., AN 92-418623, Class A23D; & JP 04-312597 A (HAYASHIBARA SEIBUTSU KAGAKU), abstract.	1-9
X	Database WPIL on Questel, week 9241, London: Derwent Publications Ltd., AN 92-337587, Class A61K; JP 04-243822A (TSUMURA & CO), abstract. -----	1-9

Form PCT/ISA/210 (continuation of second sheet) (July 1998)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/KR 98/00326

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
remark: Although claims 1-9 (as well as claim 10) are directed to a method of treatment of the human or animal body by therapy (Rule 39.1 (iv) PCT) the International Search Report has been carried out and based on the alleged effects of the composition.
2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This international Searching Authority found multiple inventions in this international application, as follows:

1. claims 1-9 concern the use of a big flavonoid of formula (I) for increasing the plasma HDL level in a mammal.
 2. claim 10 concerns the use of neohesperidin dihydrochalcone of formula (II) for increasing the plasma HDL level in a mammal.
-
1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
 2. ☐ As all searchable claims could be searched without effort justifying and additional fee, this Authority did not invite payment of any additional fee.
 3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
 4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims: it is covered by claims Nos.: 1-9

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Publication No.

PCT/KR 98/00326

Patent document cited in search report			Publication date	Patent family member(s)		Publication date
JP	A2	8283154	29-10-1996	none		
JP	A2	8280358	29-10-1996	none		
JP	A2	7061927	07-03-1995	JP	B2	2598873
						09-04-1997
JP	A2	4312597	04-11-1992	none		
JP	A2	4243822	31-08-1992	none		

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